

REVIEW

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Protein metabolism and physical training: any need for amino acid supplementation?

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Abstract

Muscle mass is the major deposit of protein molecules with dynamic turnover between net protein synthesis and degradation. In human subjects, invasive and non-invasive techniques have been applied to determine their skeletal muscle catabolism of amino acids at rest, during and after different forms of physical exercise and training. The aim of this review is to analyse the turnover flux and the relative oxidation rate of different types of muscle proteins after one bout of exercise as well as after resistance and endurance condition of training. Protein feeding in athletes appears to be a crucial nutrition necessity to promote the maintenance of muscle mass and its adaptation to the need imposed by the imposed technical requirements. In resting human individuals, the recommended protein daily allowance is about 0.8 g (dry weight) kg^{-1} body weight per 24 h knowing that humans are unable to accumulate protein stores in muscle tissues. Nevertheless, practical feeding recommendations related to regular exercise practice are proposed to athletes by different bodies in order to foster their skills and performance. This review will examine the results obtained under endurance and resistance type of exercise while consuming single or repeated doses of various ingestions of protein products (full meat, essential amino acids, specific amino acids and derivatives, vegetarian food). From the scientific literature, it appears that healthy athletes (and heavy workers) should have a common diet of 1.25 g kg^{-1} 24 h to compensate the exercise training muscle protein degradation and their resynthesis within the following hours. A nitrogen-balance assay would be recommended to avoid any excessive intake of protein. Eventually, a daily equilibrated food intake would be of primer importance versus inadequate absorption of some specific by-products.

Keywords: Muscle human proteins, Amino acid requirements, Exercise training

Background

In humans, skeletal muscle mass is the major protein molecule deposit which represents about 60% of total body protein. Besides, there is no real protein storage. Athletes and physical working subjects are therefore mainly interested to maintain this specific mass in order to keep an appropriate balance between daily breakdown and synthesis of these compulsory molecules. Muscle protein turnover is a major investigation for athletes and heavy workers under different aspects of exercise (resistance and endurance).

The major concern of this review is related to an appropriate daily requirement of protein intake versus protein degradation induced by regular exercise. The impact of exercise practice on skeletal muscle mass will

be analysed on healthy individuals. Accordingly, the benefit, or not, of specific protein and amino acid supplementation will be investigated in exercising humans. An excess of protein intake is inaccurate and costly. Appropriate advices of nutritionist/dietician might be necessary to assure an equilibrated diet.

General view of protein metabolism in humans

In human beings, the body protein mass provides architectural support, enzymes to catalyze metabolic reactions, signalling intermediates within and between cell tissues, and fuel to assume survival under extreme situations. Skeletal muscles are the major deposit of protein molecules (about 40% of body weight in young males with 20–22% body mass index, (expressed as kg.m^{-2}), and nearly 60% of total body protein in humans. Other organs or tissues contain proteins such as the liver which synthesizes plasma proteins (including albumin

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which represents nearly 50% of liver proteins), immune cells (mainly leucocytes), intestinal tract proteins (digestive enzymes), bone and dermal collagen [1]. For any cell or tissue, protein balance reflects the net protein synthesis and protein degradation which differs drastically among tissues and organs, between cell compartments.

As there is no real protein storage pool, the human body is facing a delicate and dynamic balance that maintains homeostasis facing environmental challenge. Under resting condition, steady-state measurements of fuel turnover in post-absorptive humans show a unique situation where, as compared to carbohydrates and triglycerides, proteins have the fastest turnover rate and the lowest oxidation rate (Fig. 1).

The liver and, to a lesser extent, the kidney are the principal sites of amino acid metabolism in humans. When mammals are ingesting excess protein, amino acid amounts larger than needed for synthesis of proteins and other nitrogenous compounds cannot be stored or excreted, and the surplus is oxidized or converted to carbohydrate and lipid. During amino acid degradation, the α -amino group is removed and the resulting carbon skeleton is converted into a major metabolic intermediate. Most of the amino groups of amino acids are transformed into pyruvate, acetyl-CoA or one of the intermediates of the tricarboxylic acid cycle [2].

Before starting precise details about a specific metabolic pathway, it appears mandatory to briefly introduce the kinetic structure of equilibrium and non-equilibrium reactions induced by enzymes (protein molecules) (see

references [2, 3]). Figure 2 represents the rates of forward and reverse reactions in a metabolic pathway in vivo.

A reaction in a metabolic pathway could be:

- (a) of non-equilibrium because the maximum catalytic activity of the enzyme (E1) is high in comparison to that of the reverse reaction by another enzyme (E2)
- (b) of near-equilibrium if the maximum activities of both enzymes (E1, E2) are almost identical

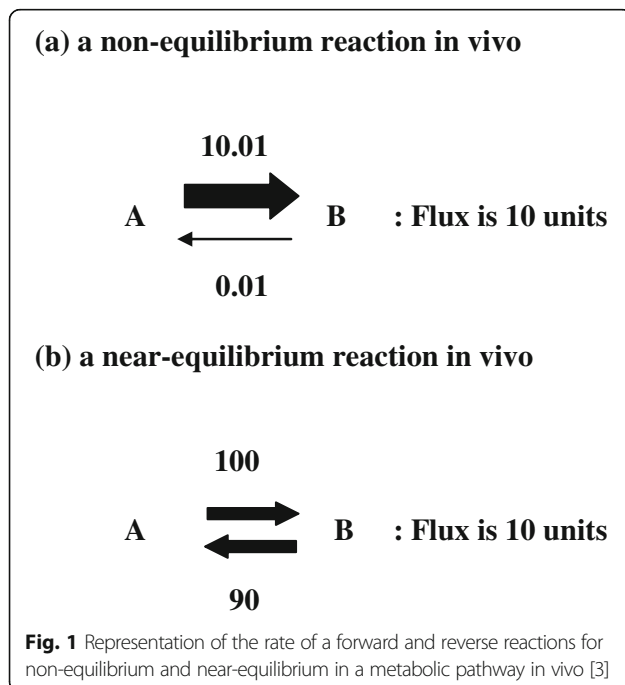
The importance of those equilibrium and non-equilibrium reactions is leading us the *flux-generating steps* in a pathway, such as glycolysis to activate the production of energy during intense exercise. The catalytic activity of flux-generating enzymes can provide a quantitative index of the maximum flux through a pathway in athletic performance (see [3] for details).

A more precise example will be the loss of the α -amino group which occurs by oxidative deamination (using the enzyme glutamate dehydrogenase) and trans-deamination (using several aminotransferases and the glutamate dehydrogenase). Most of the amino acids can be converted to their respective oxoacids by aminotransferase (also called transaminase) reactions (Fig. 3).

All but two (lysine and threonine) amino acids appear able to be transaminated although it is not always clear how large a part these reactions play in the normal degradation of amino acids in the liver. The reactions catalyzed at the aminotransferases (using pyridoxal phosphate-vitamin B6 as prosthetic group) and by glutamate dehydrogenase (using NAD^+ or NADP^+ as oxidizing agent) are close to equilibrium so that 2-oxoacids being provided, the overall process can be readily reversed and amino acids can be synthesized as well as degraded. The near-equilibrium trans-deamination system provides an easy mechanism whereby the concentrations of both amino acids and 2-oxoacids are maintained constant despite variations in the magnitude and direction of the metabolic flux through this system. The metabolism of amino acids, in addition to adenosine, generates most of the ammonia. Meanwhile, most tissues release nitrogen mainly as alanine or glutamine in order to buffer the toxicity of ammonia. The first reaction uses aminotransferase from glutamate to pyruvate, and the second reaction transfers the ammonia itself to glutamate and is catalyzed by glutamine synthetase.

Although a large proportion of the ammonia does not arise from catabolism in the liver, the urea cycle occurs exclusively in the hepatic tissue and it requires four molecules of “energy-rich” phosphate for the synthesis of one molecule of urea. In the human being as much as 90% of urinary nitrogen is in the form of urea.

The urea cycle appears to be regulated by non-equilibrium reactions with the first reaction being the flux-generating step. The synthesis of fumarate by the



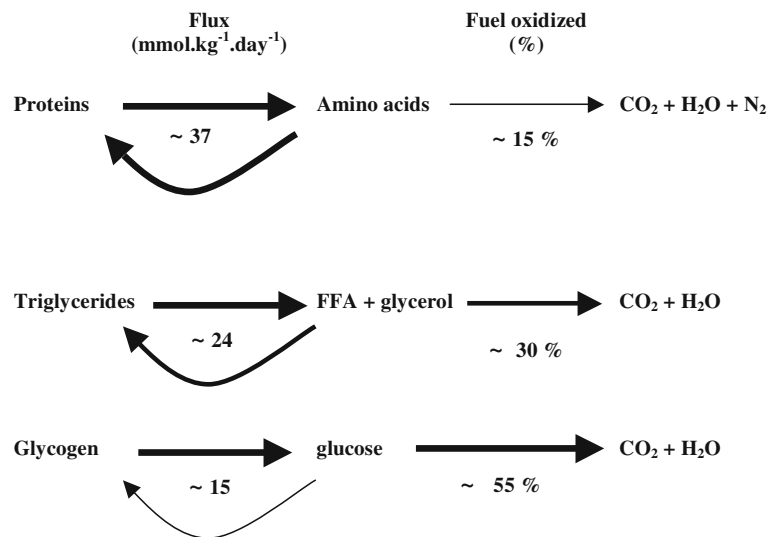


Fig. 2 Rates of daily turnover flux and relative fuel oxidation in resting individuals [58]

urea cycle is important because it links the urea cycle and the tricarboxylic acid cycle. In this respect, fumarate leading to oxaloacetate can be converted into glucose by a gluconeogenesis pathway.

Many cells are capable of concentrating amino acids from the extracellular environment, but prior to intracellular metabolism, amino acids must be transported across the cell membrane. This transport is mediated by specific amino acid transporters, proteins that recognize, bind and transport these amino acids from the extracellular medium into the cell, or vice versa [3].

The skeletal muscles, the intestines and the liver are particularly important in the disposal of excess amino acids. Much of the nitrogen is channelled into only a few compounds for the transport between tissues

(mainly alanine and glutamine). Free amino acid deposition in muscle often accounts for as much as 80% of the total amount in the whole body. In contrast, the plasma contains a very small proportion of the total amino acid pool, varying from 0.2 to 6% for individual amino acids.

The muscle free amino acid pool in normal man weighing 70 kg has been calculated to be about 86.5 g without taurine and 121.5 g with taurine [3–5]. The latter compound is synthesized from cysteine, and taurine is excreted as such or in the form of taurocholate or related bile salts. In mammalian muscle tissues, taurine acts as a membrane stabilizer and a modulator of the Ca²⁺ storage capacity of the sarcoplasmic reticulum. Of the total pool of human skeletal muscle free amino acids, the eight essential amino acids represent only

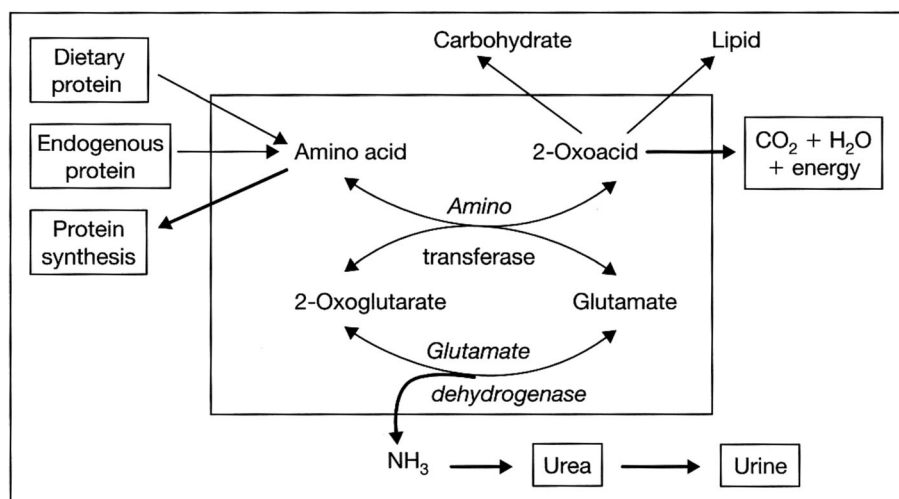
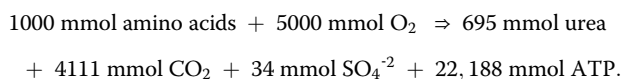


Fig. 3 Central role of trans-deamination in metabolism of amino acids (adapted from [3])

8.4% whereas glutamine, glutamate and alanine constitute nearly 79% (Table 1). Among the amino acids the branched-chain amino acids (BCAA) (leucine, isoleucine and valine) are of particular interest as 60% of the total distribution of specific enzymes necessary for their oxidation (α -keto acid dehydrogenases) in man are located in skeletal muscle. These amino acids, unlike most of the others, are taken up by the striated muscles after a meal and partially oxidized in those tissues. In the postabsorptive period of starvation, the leg muscle of man releases essentially alanine and glutamine (60% of the total release) [3].

Amino acid oxidation in muscle leads to an appreciable amount of ATP generation. McGilvery nicely proposed a total balance of amino acid degradation when a human being consumes 530 g of raw lean meat (110 g of protein or a mean 1000 mmol of amino acids) [6, 7]. Assuming a common composition for the amino acids in muscle, the total balance of amino acid degradation is:



This equation also states that about 0.4 mol equivalent of glucose can be formed in the liver, or 72 g from the 110 g of mixed amino acids. The complete oxidation of leucine, isoleucine and valine gives 43, 42 and 32 mol of ATP, respectively, per mole of each amino acid. However, the overall P/O ratio is only 2.2, compared to 2.8 for fats and 3.1 for glycogen, so amino acids are not a good fuel for maximum power production.

At present, especially in humans, it is rather difficult to estimate precisely in skeletal muscle the energy balance from the daily supply of amino acids. However, arterio-venous differences in amino acids across leg muscle in post-absorption condition reveal that nearly 70% are released as glutamine (30%), alanine (30%) and glycine (10%) [3].

Jungas et al. [8] calculated the net ATP and acid-base balances associated with amino acid oxidation in the skeletal muscle. These authors concluded that the overall ATP balance under resting condition amounts to ~4500 mmol excess ATP per day or about 50% of

the total oxidation from amino acids in the muscle, small intestine, kidney and liver tissues taken together. Again, this emphasizes the importance of muscle mass in the energy balance of the whole organism. The net effect of the oxidation of amino acids to glucose on the liver is to make nearly two thirds of the total energy available from the oxidation of amino acids accessible to peripheral tissues.

Protein turnover in resting individuals

General view

The term turnover covers both the synthesis and breakdown of protein. In the steady state condition, the energy cost of protein synthesis will approximately account for 10% of the basal oxygen uptake [9]. Skeletal muscle turnover is regulated in part by nutrition, as dietary energy intake and macronutrient distribution, especially by the quality and quantity of dietary protein and amino acids, influence muscle protein breakdown and synthesis [10]. Total protein synthesis in human adult subjects is about $3.0 \text{ g.kg}^{-1}.\text{day}^{-1}$ [11] while protein turnover is about $5.7 \text{ g.kg}^{-1}.\text{day}^{-1}$ [9]. Protein degradation in human skeletal muscles estimated from the release of tyrosine in the presence of insulin and amino acids is approximately $34 \text{ nmol.h}^{-1}.\text{g}^{-1}$ wet weight. This degradation rate corresponds to a half-life of approximately 20 days [12].

Protein digestion is a complex process that involves dynamic movements and exchanges of peptides, amino acids and ammonia between the gut lumen and different systemic pools. The optimal intakes of whole protein in the human diet have been a matter of debate for many years [13–16]. Apparently, the nitrogen balance data for healthy adult men and women rose from 0.60 g kg^{-1} per 24 h in 1979 to 0.80 g kg^{-1} per 24 h. Urinary nitrogen is a particularly reliable marker of protein intake [17]. However, the recommended intakes of protein are higher in young children ($1.2 \text{ g.kg}^{-1}.\text{day}^{-1}$ at 1 year) and slowly decrease in resting young adult state (18 years). Vegetarians restrict their diet to plant food, and those individuals may be at risk of not getting adequate amounts of some indispensable amino acids (lysine, methionine, cysteine and threonine) because of their inadequate amounts in plant food proteins compared to animal proteins. Moreover, plant proteins are generally less digestible than animal proteins. Nevertheless, available evidence does not support recommendation for separate protein requirement for vegetarians who consume complementary mixtures of plant protein [13].

As illustrated in Fig. 3, ammonia is produced when the muscle does work. This production is proportional to the work done [18–21]. This ammonia is delivered by the skeletal muscle from the purine nucleotide cycle during short-term and long-term intense physical activities.

Table 1 Average values of specific protein fractional synthesis rates (FSR) in human skeletal muscle (fasted state). Adapted from Guillet C et al [1]

Muscle fractions	FSR, mean \pm SD (% per day)
Myosin heavy chain	0.90 ± 0.08
Actin	1.80 ± 0.19
Sarcoplasm	1.29 ± 0.20
Mitochondria	1.94 ± 0.10

Deamination of amino acids is a likely source of ammonia during prolonged exercise.

We shall focus our interest mainly on the skeletal muscle and collagen tissues (tendon) which appear to be quantitatively most important for physically active people.

Skeletal muscle tissues

The skeletal muscle tissue contains a few thousands of specific proteins which could be distributed as myofibrillar, sarcoplasmic and mitochondrial fractions. The myofibrillar proteins are different molecules such as myosin heavy and light chains, actin, tropomyosin, troponins (T, I and C), titin, elastin, Sarcoplasmic proteins (which represent about 20–30% of total muscle proteins) are made of glycolytic enzymes, proteins of the sarcoplasmic reticulum (calsequestrine, calcium-ATPase, ...). Mitochondrial proteins of muscle tissues consist of enzymes of tricarboxylic acid cycle, β -oxidation, and respiratory chain. The synthetic rates of myosin are lower than those of other muscle fractions (Table 1).

Thus, it appears that the fractional synthetic rate (FSR) of actin is more or less twofold that of the myosin heavy chain. The precise mechanism of this specificity remains unknown up to now. The overall control of the size of the human skeletal muscle mass has been elegantly reviewed by [22]. Eventually, Bohé et al. concluded that the rates of synthesis of all class of muscle proteins (mixed, myofibrillar, sarcoplasmic and mitochondrial) are acutely regulated by the blood essential amino acid concentration over their normal diurnal range, but become saturated at high concentrations [23]. Thus, the stimulation of protein synthesis depends on the sensing of the concentration of extracellular, rather than intramuscular essential amino acids.

Collagen tissues

Extracellular matrix (ECM) placed in tendon tissue ensures a functional link between the skeletal muscle mass and the bone. The ECM molecules consist of a variety of glycoproteins of which the major part consist of proteoglycans collagen fibrils, the latter one being predominant (60–85%) (see [24]). The fundamental building block of collagen fibrils are formed by three polypeptide α -chains that compose a triple helical structure. Collagen is 35% glycine, 21% proline and hydroxyproline and 11% alanine. This unusual amino acid content is imposed by structural constraints unique to collagen molecules.

Collagen is the most abundant single protein in most vertebrates (humans included), up to nearly a third of the total protein mass. Collagen molecules are not synthesised in the muscle tissue but in fibroblasts which are scattered within the tissue. Given the importance of collagen to the skeletal muscle function, the knowledge of its quantitative synthetic rate was either ignored or estimated to be very slow. These

last years, a few studies revealed that FSR of the patellar and Achilles tendon collagen amounted to a mean value of 1.08% per day in resting men, nearly 30% less in women [25]. Comparing the FSR of collagen and myofibrillar proteins in humans, there is no major difference as supposed previously but muscle collagen is not at all responsive to feeding [26]. Growth hormone (GH) and recombinant human GH have no effect on human muscle size and muscle protein synthesis (MPS) [27] but do have a positive effect on strengthening the collagen matrix in musculotendinous tissue [28].

Specific effects of exercise on muscle protein content

There are a few review publications related to the regulation of human muscle protein synthesis and breakdown during and after resistance exercise [29–36]. We will have to differentiate the results obtained in fasted (post-absorptive condition) or fed state, during or after exercise, for muscle protein synthesis (MPS) or muscle protein breakdown (MPB).

Data in Table 2 clearly demonstrates that short-term intense resistance exercises induce a higher mixed skeletal muscle protein increase after stopping the exercise. The

Table 2 Effect of resistance exercise on total muscle protein synthesis (MPS) and muscle protein breakdown (MPB) under untrained condition and in fasted state

Exercise protocol	FSR (% \cdot h $^{-1}$)	FBR (% \cdot h $^{-1}$)	Reference
	PostEx/PreEx ratio	PostEx/PreEx ratio	
Mixed muscle proteins			
4 x 6-12 rep. 80% max	+49%*	-	[158]
5 x 10 rep. 100 max	+136%*	-	[112]
8 x 8 rep. 80% max	+140%*	+36%*	[159]
8 x 120% max	+122%*	+40%*	[160]
6 x 8 rep. 80% max	+30%*	-	[161]
8 x 10 rep. 75% max	+36%*	NS	[162]
10x10 rep. 80% max	+50%*	-	[163]
4 x 10 rep. 80% max	+135%*	-	[164]
5 x 90% max	+350%*	-	[37]
Myofibrillar proteins			
5 x 90% max	+330%*	-	[37]
6 sec at 30% max to exhaust.	+175%*	-	[38]
Sarcoplasmic proteins			
5 x 90% max	+77%*	-	[37]
Mitochondrial proteins			
6 sec at 30% max to exhaust.	+175%*	-	[38]

MPS and MPB are expressed, respectively by their fractional synthetic rate (FSR) and fractional breakdown rate (FBR), rep. = repetitions, max = % of maximal oxygen uptake, exhaust. = up to exhaustion, PreEx = pre-exercise, PostEx = postexercise
* = significant ($P < 0.05$), NS = not significant between pre- and post-exercise

varied effects between the reports may result in methodological differences (see [33]) but generally speaking, the highest benefit seems to be linked to the total work output (80–90% of maximal contraction). Resistance exercise seems to be more efficient on myofibrillar proteins compared to sarcoplasmic proteins [37, 38]. Moreover, it appears that those increases in muscle protein synthesis remain up to 4 h after stopping the exercise. On the contrary, resistance exercise does not seem to have major effect on muscle protein breakdown.

The effects of feeding a protein mixed meal under resting condition doubles muscle protein synthesis (see [29, 39–41]). Several publications reported the effects of resistance exercise on human muscle protein synthesis and breakdown (Table 3).

The ingestion of protein immediately before the start of exercise or during resistance exercise has no effect on muscle mass and strength in young adults [36, 42–44]. Protein feeding has been applied essentially after stopping the exercise. In most, if not all conditions, muscle protein synthesis has been enhanced by ingestion of different supply of amino acids. The increase in MPS is observed in mixed muscle, myofibrillar and sarcoplasmic fractions. The enhanced amount of muscle proteins depends on the quantity of ingested portion, the relative proportion of essential amino acids (EAA) being either supplemented as free EAA or as major portion of whey

proteins (about 50% of EAA). It appears that MPS is increased when the AA are ingested immediately after stopping the exercise session [45]. Rapid aminoacidemia in the post-exercise period enhances MPS and the anabolic signals leading to the increase in muscle protein mass. Moreover, it seems that a bolus of 25 g dose is more efficient than a series of small pulsed drinks (10×2.5 g) [46]. Both myofibrillar and sarcoplasmic proteins may remain stimulated up to 3–5 h post-exercise [45, 47, 48] or even up to 24 h in young men when the intensity of exercise is high [46, 49].

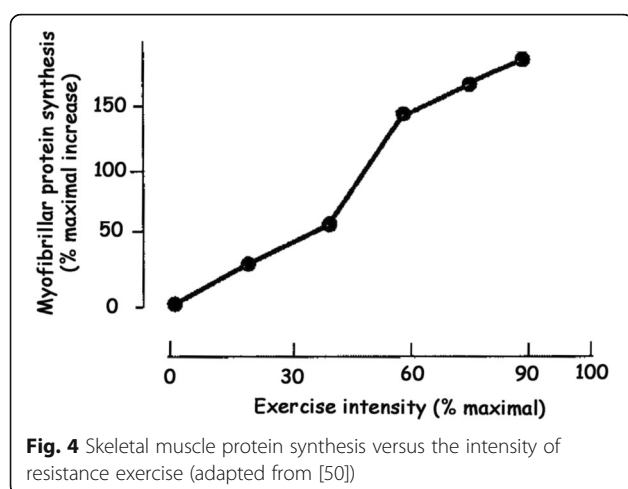
In order to stimulate the skeletal muscle synthesis, it is of prime importance to increase the power output during the exercise session (Fig. 4). It seems that the optimal synthetic rate is attained by nearly 80–90% maximal resistance exercise in young healthy subjects [50].

There is a general consensus about the dietary protein requirements to optimum adaptation for athletes: daily intake in the range of 1.2–1.8 g (dry weight).kg⁻¹ body weight [31, 34, 36, 51]. Moreover, knowing that the human organism is unable to accumulate protein stores (such as fat depots), we demonstrate that daily excess protein intake enhances whole nitrogen balance in healthy athletes [51]. The net nitrogen balance in male [51] and female [52] athletes is attained at a mean protein daily intake of 1.25–1.28 g.kg⁻¹ body weight.day⁻¹.

Table 3 Effects of resistance exercise on human MPS and MPB in the fed state (selected references)

Exercise protocol	Nutritional protocol	FSR (%.h ⁻¹)	FBR (%.h ⁻¹)	Reference
		PostEx/PreEx ratio	PostEx/PreEx ratio	
Mixed muscle proteins				
5 x 10 rep. max	10g AA (IV)	+121%*	NS	[165]
10 x 8 rep. 80% max	6g EAA (oral)	+340%*	NS	[166]
4 x 10 rep. 80% max	10g whey +CHO (oral)	+130%*	-	[167]
10 x 10 rep. 70% max	Leu EAA + CHO (oral)	+167%*	-	[113]
4 x 10 rep. max	40g egg proteins (oral)	+90%*	-	[168]
8 x 10 rep. 70% max	10G whey (oral)	+33%	*	[169]
Myofibrillar proteins				
5 x 10 rep. 80% max	1g protein.kg ⁻¹ (oral)	+83%*	-	[53]
20 x 10 rep. 75% max	6g protein.h ⁻¹ (oral)	+188%*	NS	[170]
stepping ex (+25% bw)	45g EAA + CHO	+221%*	-	[171]
5 x 10 rep. max	25g whey (oral)	+229%*	-	[104]
8 x 10 rep. max	25g whey (oral)	+193%*	-	[47]
10 x 8 rep. 80% max	0.3g.kg ⁻¹ LM whey	+90%*	-	[45]
4 x 10 rep. 80% max	20g whey protein (oral)	+48%*	-	[49]
Sarcoplasmic proteins				
20 x 10 rep. 75% max	6g protein.h ⁻¹ (oral)	+300%*	-	[170]
5 x 10 rep. max	25g whey (oral)	+104%*	-	[48]

AA = amino acids, EAA = essential amino acids, CHO = carbohydrate, NS = non -significant, * = $P < 0.05$



Effect of exercise training on the muscle protein synthesis and breakdown in humans

According to the review paper of Kumar et al., it appears that chronic resistance exercise increases mean the muscle fibre cross-sectional area and provokes muscle hypertrophy [29]. Several authors reported an enhanced basal rate of MPS, but it seems difficult to have a precise idea about those changes due to the lack of information on the time course of the last bout of exercise sessions during the training schedule. However, an accurate report before and after 10-week training indicated an increase in the basal synthesis of myofibrillar proteins under resistance exercise while endurance training enhanced basal mitochondrial protein synthesis [53]. Collagen synthesis is similar in the muscle after eccentric and concentric exercise training [54].

Sex differences in muscle protein metabolism under exercise condition

The scientific literature does not give us major information about a lower muscle mass in women as compared to men, besides anabolic hormonal intervention, such as testosterone. Vingren et al. speculated about the differential effects of several hormones, such as gonadotrophin releasing hormone and adrenocorticotrophic hormone, which could explain the muscle mass sex difference [55]. They concluded that testosterone plays only a minor role to explain the difference of the muscle mass between women and men. Moreover, Kumar et al. did not report differences in the basal or post-exercise rates of MPS or MPB between young men and young women [29]. As well, using two variable protein intakes, Pannemans et al. did observe identical nitrogen balance and whole-body protein turnover in young men and women [56]. However, postmenopausal women have about 20–30% higher basal rates of MPS than men [57].

Thus, we are still looking to further investigate about the differential mechanisms.

Dietary protein requirements to optimum adaptation in resistance athletes

A meta-analysis of 23 publications gives evidence that protein supplementation augments the adaptative response of the skeletal muscle to resistance-type exercise training [35]. However, maximizing the rate of muscle protein synthesis depends on the type of dietary protein sources and the timing of intake of protein-rich foods to increase its effect on athletes. Several techniques have been proposed to stimulate protein synthesis before, during and after resistance exercises: food from meat, milk, whey, essential amino acids (EAA), branched-chain amino acids (BCAA) and leucine [31, 36, 44]. As humans need to ingest the eight essential amino acids (from beef, fish, milk, vegetables) to covert the synthesis of their own cellular protein molecules, athletes have to remain vigilant about their food-specific intake. This can be rather easy for omnivorous and vegetarian individuals, but more tricky for vegan athletes.

Mechanisms leading to the regulation of muscle protein synthesis

Muscle protein synthesis and degradation are regulated by hormonal and nutritional factors [22, 58]. Those factors are acting on the sarcolemma receptors and sarcoplasmic effectors which promote the activation of translational initiation of protein synthesis.

Hormonal implications

Basically, four main hormones appear to be the major effectors acting on body protein metabolism: insulin, insulin-growth factor-1 (IGF-1), testosterone and growth hormone (GH). It is commonly reported that resistance exercise with moderate to high intensity and volume induces the blood release of IGF-1, testosterone and GH. However, as said previously, the exact role of testosterone in resistance training programme is still hard to pinpoint [55]. But an elegant report of West et al. reveals that transient resistance exercise induces intramuscular signalling responses, together with post-exercise muscle protein synthesis [47]. However, Phillips estimated that anabolic hormone intervention in the adaptation of MPS after resistance exercise is more likely as “chasing a hormonal ghost” [59]. Thus, other local intramuscular mechanisms appear to monitor the acute effect of resistance post-exercise MPS response.

Regulatory mechanisms of skeletal muscle protein turnover during exercise

Two major signalling pathways control myofibrillar protein synthesis: (1) the insulin-like growth factor 1 (IGF1)-

Akt-mammalian target of rapamycin (mTOR) pathway acting as a positive regulator [60, 61]; (2) the myostatin acting as a negative regulator to avoid a deregulated processes [60]. Additionally, the activation of a protein named peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) contributes to mitochondrial content and biogenesis [62, 63]. Figure 5 summarises these general signalling pathways.

Protein synthesis is regulated by the IGF-1 and a cascade of intracellular effectors that mediate muscle hypertrophy. Among the numerous effects induced by exercise, the Akt-mTOR pathway is known to promote muscle growth [60, 61], in addition to the nervous stimulation at the skeletal muscle membrane that induces the release of calcium from the sarcoplasmic reticulum. Most of these effectors are positively controlled by phosphorylation mechanisms leading to muscle fibre hypertrophy and mitochondrial biogenesis (including some regulatory enzymes).

Mechanical deformation of skeletal muscle fibres induced by muscle contraction stimulates several signals included in the sarcoplasm [29, 53, 58, 64–68]. Among those regulators acting on gene expression, one can identify amino acids [69–71], AMP-activated protein kinase (AMPK) [72], mammalian target of rapamycin (mTORC1) [64, 65, 73, 74] and mitogen-activated protein kinase (MAPK) [65].

The essential amino acids, mainly leucine [65, 70, 75] and glutamine [71], the most abundant muscle amino acid, are acting on several kinases to stimulate the translation initiation of protein synthesis. The ingestion of dietary amino acids after exercise alters the phosphorylation state of several regulatory proteins (mTORC1 and MAPK)

leading to increased myofibrillar protein synthesis after resistance exercise training and regulatory oxidative enzymes during endurance training.

Acute changes in protein synthesis are primarily regulated at the level of mRNA translation via translational efficiency [65, 76] (Fig. 6). Non-coding RNA, called microRNAs (miRNAs), control the development, function and adaptation of the skeletal muscle [77–80] through a posttranscriptional mechanism involving inhibition of translation and/or degradation of mRNA transcript. Several studies show that exercise is capable of regulating miRNA levels. They have a central role in skeletal muscle plasticity [81–84].

The miRNAs are defined as 21–30 small single stranded non-coding RNAs produced from hairpin-shaped precursors [85, 86]. From a microRNA gene, a primary-miRNA (pri-miRNA) is initially transcribed by RNA polymerase II in the nucleus as long primary transcripts of several kilobases. Then, a RNA II endonuclease cleaves the pri-miRNA into a 60–70 nucleotide (pre-miRNAs). An Exportin-5-GTP transports the pre-miRNA from the nucleus to the sarcoplasm where it is cut by a RNA III enzyme into a 22 nucleotide mature miRNA. Skeletal and cardiac muscles are highly enriched in several miRNAs, named myomiR (miR). The miR-206 is a unique amount of the myomiR family in that it is specifically expressed in the skeletal muscle. Kim et al. suggest that miRNA-206 negatively regulates DNA polymerase translation, thereby inhibiting DNA synthesis [87]. Thus, these myomiR could block the formation of the skeletal muscle mass. Moreover, it is postulated that miR-206 has an important role in regulating the expression of genes involved in satellite cell specification during fibre type transitions in the muscle [78].

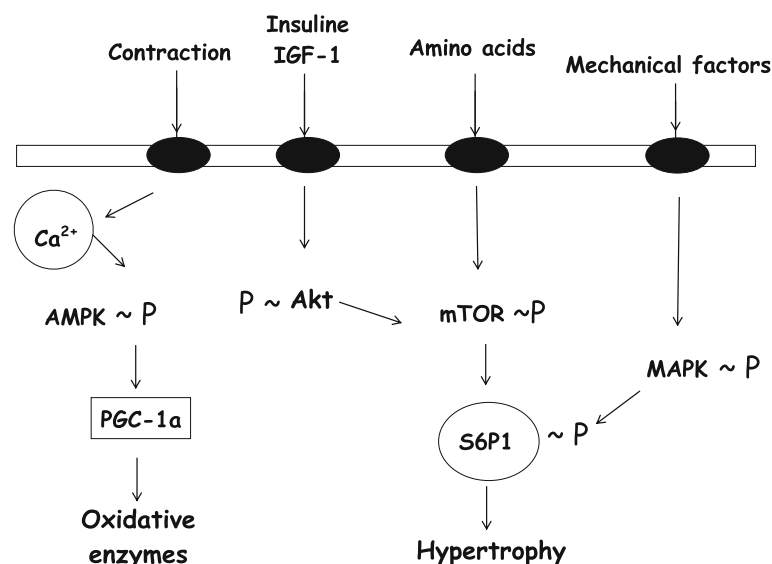


Fig. 5 Signalling pathways for skeletal muscle normal growth and induced hypertrophy

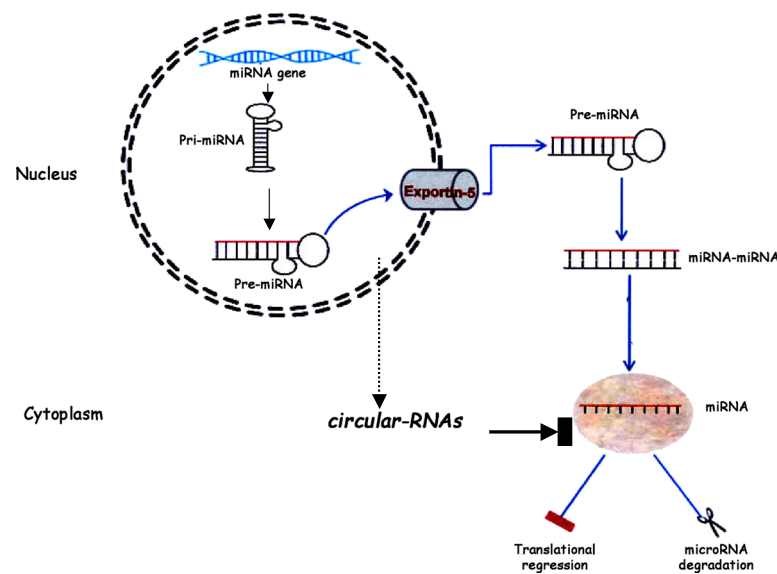


Fig. 6 Schematic view of micro-RNAs processing in the skeletal muscle

A few publications on miRNA have been released on the effect of resistance exercise training on human subjects [88, 89]. McCarthy and Esser reported that the expression of two miRNA were downregulated by 50% following 7 days of skeletal muscle hypertrophy exercise [88]. However, Davidson et al. could not confirm this observation to all subjects after 12 weeks of resistance exercise training [89]. Some subjects were “low” responders (about 50% reduction of several miRNAs) while others (“high” responders) failed to modulate their genes. The “high” and “low” responders of microRNA expression in the skeletal muscle might be explained by a different reaction to resistance training as compared to other subjects [89]. Eventually, an important question remains: what is regulating myomiRNA transcription? The answer(s) could be linked to a recent discovery; circular-microRNAs that regulate the synthesis of the so-called microRNAs, thus acting to stimulate or refrain the synthesis of new protein molecules [90].

There is compelling evidence that genetic factors influence several phenotype traits related to physical performance and training response as well as elite athletic status [91]. Moreover, complex regulation can modulate gene expression by epigenetic mechanisms such as DNA methylation and histone modification with persistent effects on the availability of DNA for transcription into protein molecules (see review by [92]). Therefore, future investigations should extend our knowledge on epigenetic effects that could play a “reasonable” role in the determination of athletic potential. As suggested many years ago by the late famous Swedish scientist, Professor P.O. Astrand, “we ought to chose our parents!”

Practical feeding recommendations for regular exercise practice

The information given herewith fosters the attention of athletes or regular exercising individuals to take care of adequate protein feeding to maintain or increase their skeletal muscle mass status. However, the scientific literature reveals a wide variety of practical conducts which promote the adaptation of muscle mass through specific food applications: how much, with or without carbohydrates, what type of protein, how, when? We shall try to separate the wheat from the chaff.

The World Health Organization (WHO; the USA Institute of Medicine, France and Belgium health organizations) established precise rules related to the recommended daily protein allowance (RDA) of young sedentary adults [13]: 0.83 g.kg^{-1} body weight. Taking the statistical distribution in adult subjects, there will be an alimentary deficit of protein intake when less than $0.40\text{--}0.50 \text{ g.kg}^{-1}$ body weight [93].

Nevertheless, the daily load of $\pm 0.8 \text{ g.kg}^{-1}$ body weight appears insufficient for adults practising regular physical activities of medium or high intensity (leisure, sports, working professions). Numerous publications do suggest a slightly regular increase above the “RDA” amount [2, 14, 34, 36, 94–103].

How much proteins?

There is mounting evidence that the timing of ingestion and the protein source during recovery influence the extent of muscle hypertrophy [104]. Minor difference in muscle protein turnover appears to exist between young men and women. Adequate protein balance is the result

from an equation between the quantity of protein ingested per day and the amount of protein utilized under exercise condition. As detailed previously, one could estimate this balance using the N intake by food and release by the N wastes (mainly in urine). The nitrogen balance (NBal) has been utilized since a long period (see [2]), even if one estimate, this is not the most accurate method. However, it remains an indirect method to evaluate the daily balance between protein intake by daily food questionnaire and nitrogen release from protein degradation (mainly muscle mass) by urine collection. Table 4 gives an example of NBal recorded on young athletes (Poortmans unpublished data).

Figure 7 shows the scatter distribution of NBal among young orienteering athletes and bodybuilders engaged in regular training. It appears that a general adult population under exercise condition could easily equilibrate its NBal with a mean daily intake of $1.25 \text{ g protein}^{-1} \cdot 24 \text{ h}^{-1}$. In one study on young gymnasts, using both NBal and ^{15}N -glycine technique, we were able to observe a positive net protein balance ($+0.61 \text{ g protein}^{-1} \cdot 24 \text{ h}^{-1}$) with a mean protein intake of over $1.39 \text{ g protein}^{-1} \cdot 24 \text{ h}^{-1}$ during a training season [105]. Additional investigations on whole body protein turnover [106] and skeletal muscle fractional synthetic rates in trained endurance humans [107] suggest that a protein intake of $1.2 \text{ g protein}^{-1} \cdot 24 \text{ h}^{-1}$ (or 10–12% of total energy) should achieve a positive nitrogen balance. A recent survey by Slater and Phillips reported protein intake among adult male strength and power athletes during their training [108]. The recorded quantity of protein intake amounts from 1.1 to $3.3 \text{ g protein}^{-1} \cdot 24 \text{ h}^{-1}$ [109]. However, as said above, even in those strength athletes, there is no real evidence to absorb more than $1.25 \text{ g protein}^{-1} \cdot 24 \text{ h}^{-1}$.

How much protein is safe? A daily amount of 8–12% of protein intake seems to be adequate over the whole range of life appears adequate and well balanced [110]. But would an excess of protein and amino acid intake have detrimental effects on the human organism? Already in 1981, Waterlow and Jackson stated that excess dietary is immediately oxidized [9]. Probably for most nephrologists and internal medicine practitioners. Consumption of high-protein diets in humans may have relevance to the occurrence of osteoporosis and hypercalciuria [15]. We evaluated the consequences of excess protein intake on glomerular filtration rate (creatinine clearance), glomerular membrane permeability (albumin urine excretion) and calcium metabolism (calcium urine excretion rate) [51]. Protein intake under a mean $2.8 \text{ g protein}^{-1} \cdot 24 \text{ h}^{-1}$ does not impair renal function in well-trained athletes as indicated by the measures of renal function. But all excess of protein intake will be a waste of money and a higher nitrogen excess (essentially urea) on the organism. Protein supplementation under exercise condition should be addressed to stimulate net muscle protein synthesis, and more specifically the optimal proportion of essential amino acids [93, 111].

With or without added carbohydrate?

It has been reported that hyperinsulinemia stimulates rates of muscle protein synthesis [112–115] and inhibits protein breakdown [116], leading to protein accretion. A post-exercise feeding strategy that provides $1.2 \text{ g carbohydrate} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ seemed to improve the muscle fractional synthetic rate by 60% [115], but another study concluded that CHO does not augment exercise-induced protein accretion versus protein alone [117]. The current literature remains equivocal in terms of post-exercise protein accretion, with or without CHO addition. A recent

Table 4 N balance (NBal) of athletes recorded using a food questionnaire (over a 7 day survey) and urine nitrogen determination (twice 24h)

Athletes (number)	Gender	Age (years)	NBal (mean \pm SE) * (g.protein $^{-1} \cdot 24\text{h}^{-1}$)	NBal > than 0 ** (g.protein $^{-1} \cdot 24\text{h}^{-1}$)
Running (5)	M	19-23	1.46 ± 0.07	1.22
Rowing (10)	M	15-20	1.28 ± 0.07	1.15
Cycling (12)	M	17-21	1.59 ± 0.09	1.37
Swimming (27)	M, F	11-18	1.52 ± 0.14	1.50
Gymnastic (13)	F	8-11	1.61 ± 0.36	1.39
Gymnastic (9)	F	15-16	1.12 ± 0.18	0.85
Basket-ball (14)	M	19-39	1.74 ± 0.13	1.18
Aerobic (16)	F	19-33	1.23 ± 0.05	1.17
Orienteering (15)	M	22-34	1.35 ± 0.12	1.30
Bodybuilding (19)	M	25-36	1.94 ± 0.13	1.30

M = male, F = female; * Measured NBal from food questionnaire and total urine nitrogen, ** Positive NBAL for all subjects investigated

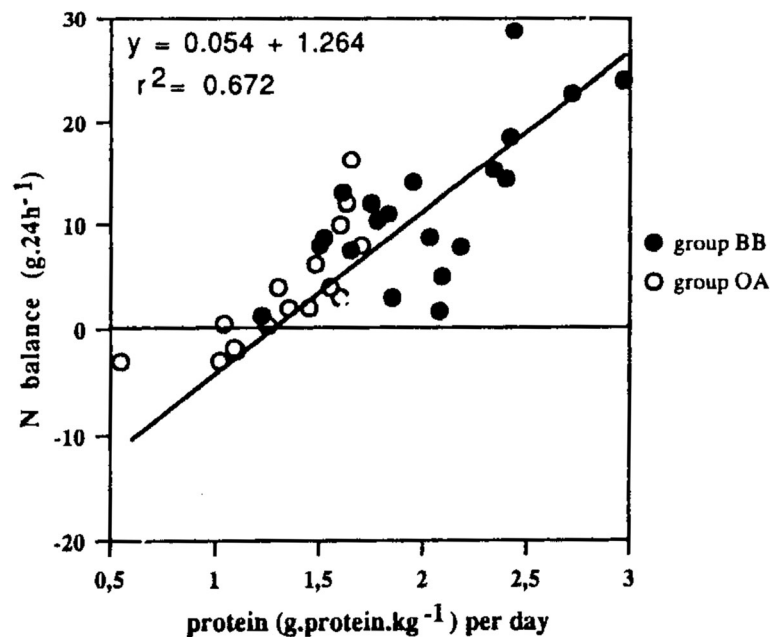


Fig. 7 Distribution of NBal among basketball players (*full dots*) and orienteering runners (*open dots*) over 1-week survey) [51]

prevailing-challenging view has been proposed to reach a conclusion statement [118]. To sum up that statement, it can be said that athletes involved in regular training could add some CHO to their protein supplement since they have to keep a balanced diet to replenish both their glycogen store and stimulate their muscle protein accretion. Nevertheless, another statement argues that addition of carbohydrate to essential amino acid mixture does not require such additional energy [119] !

What type of protein to ingest?

Animal or plant protein, all 20 amino acids, essential amino acids, and single leucine have been supplemented under resting conditions and mainly after exercise. While resting, Boirie et al. demonstrate that dietary amino acid absorption is faster with whey protein than with casein [120], but there are no differential metabolic effects on skeletal muscle breakdown and synthesis when comparing feeding with casein or soy protein [121]. As mentioned earlier, supplementation during exercise does not act on protein synthesis [122]. But there is a total consensus that feeding in the recovery period from exercise induces muscle protein accretion. Let us remember, once more, that the skeletal muscle represents about 40% of total mass and that the three branched-chain amino acids (BCAA) (leucine, isoleucine and valine) are mainly taken up by the skeletal muscles from protein eaten in fasting condition [7].

As a whole, the World Health Organization (WHO) proposed a daily protein intake of about 40% of mainly animal origin while vegetarians should add some 10% due

to the fact that intestinal absorption of plant proteins seems less adequate.

Endurance type of training Bolster et al. investigated endurance athletes who consumed three different protein intake (light 0.8 g.kg⁻¹ body weight.day; medium 1.8 g.kg⁻¹; high 3.6 g.kg⁻¹) [107]. After exercise, there was no relationship of protein synthesis according to the food intake. Nevertheless, Di Donato et al. slightly modulated this conclusion when looking at young untrained subjects under fast condition practising 60 min at 30% Wmax, or 30 min at 60% Wmax on bicycle [123]. In both cases, they observed a 60% myofibrillar protein increase of the vastus lateralis muscle in the post-exercise phase together with a stable mitochondrial protein fraction. Moreover, these authors observed a maintenance of protein synthesis of the two muscle compartments up to 24–48 h post-exercise.

A few publications pointed out the consequence of different protein intake among trained endurance athletes (Table 5).

Table 5 seems to lead to the conclusion that higher protein intake does not have “magic” influence upon endurance training. Nevertheless, an adequate reasonable daily intake of protein (see above) has a positive impact related to exercise performance.

However, an excess of protein intake or an abusive supplementation of amino acid intake has no real interest for endurance athletes. Indeed, all excess of protein consumption need to be oxidized by the liver

Table 5 Muscle protein synthesis in trained endurance athletes after different proportions of protein intake while on endurance training at 50–75% VO_2max . Total protein intake (Pro), milk, essential amino acids (EAA) or leucine (Leu)

Types of food	Quantities	Protein synthesis	Authors
Pro	2,5g.kg ⁻¹	stable	[172]
	0,22g.kg ⁻¹	stable	[173]
Whole milk	4 ml.kg ⁻¹	stable	[174]
	0,092g.kg ⁻¹	+100%	[175]
EAA	13,3g	+12%	[176]
Leu	45 mg.kg ⁻¹	+13%	[177]
	6g	stable	[178]

as demonstrated by a net positive nitrogen balance (see Fig. 7).

Effects of resistance training Some recent reviews (2012–2016) related to resistance training in humans clearly demonstrated a positive impact of daily protein intake upon myofibrillar synthesis [35, 36, 50, 99, 101, 102, 123–127] (see Table 6). Let us also state that the BCAA represents nearly 46% of the essential amino acids required per day [7].

a) Classical protein feeding

Two publications give us information about one single strength exercise either after daily protein intake of 1.2 g.kg⁻¹ body weight.day [128] or after an increasing load of beef meat of 0.7 to 2.1 g.kg⁻¹ body weight.day [129]. The first authors did not observed any modification of muscle stem cells while the second authors mentioned that one single session of resistance exercise slightly improved myofibrillar protein synthesis with an intake of 2.1 g.kg⁻¹ body weight.day.

b) Milk and derivatives

As a point of view, full or skimmed milk contains two major types of proteins: casein and whey proteins (milk without casein). It appears that whey proteins are faster (twice) rejected from the stomach into the duodenum as compared to casein [130–133] increasing therefore food availability during muscle exercise. As well, Burke et al. observed that whey protein ingestion induced a faster inclusion within plasma volume as compared to whole milk [44]. Moreover, Hansen et al. realized that whey protein ingestion had a higher benefit to perform a 4-km orienteering running (–17 to 26 s), in addition to a reduced plasma markers of several cytokines (ILs, TNF α) and muscle proteins release (creatine kinase, myoglobin) [134]. Eventually, Phillips et al. reported

the importance of milk derivatives such as calcium, potassium and vitamin D in addition to protein content [102].

c) Essential amino acids (EAA) and derivatives

Among the eight EAA, the branched-chain amino acids (BCAA), leucine, isoleucine and valine, are mainly stored within the muscles and it appears that leucine has a major stimulating role in muscle protein synthesis. Thus, supplementation of EAA, and more specifically leucine, is mandatory to muscle protein synthesis specifically by liquid disposal [31, 124, 135, 136]. Moreover, supplementation of EAA (15 g, twice a day, during 12 weeks) induced a 3.3% of muscle mass (gastrocnemius) as compared to a placebo group (+2.3%) [137].

Other molecules derived from amino acids have been used to boost athletic performance such as L-citrulline (a precursor of arginine) (a), β -alanine (issued from carnosine) (b) and taurine (synthesised from cysteine) (c).

(a) L-citrulline [138]: daily supplementation of 6 g.kg⁻¹ body weight.day during 1 week enhances maximal power (+14% W) and sustained time in seconds (+13%)

(b) β -alanine [139]: derived from carnosine (a dipeptide), the use of β -alanine was tested 10 years ago in

Table 6 Muscle protein synthesis in human subjects submitted to resistance (strength) training under different supplementation of protein or related substances. Pro (total protein), beef, whey protein, casein, soja, essential amino acids (EAA), leucine

Types of food	Quantities	Protein synthesis	Authors
Beef	2,5g.kg ⁻¹	+50%	[129]
Animal protein	0,2.kg ⁻¹ .h ⁻¹	+50%	[179]
	1,18g.kg ⁻¹	stable	[128]
Whey proteins	20g	+84%	[50]
	25g	+65%	[42]
	20g	+64%	[180]
	80g	+48%	[49]
	18g	+38%	[169]
	20g	+49%	[181]
Casein	17g	+145%	[155]
	27g	+6%	[128]
EAA	0,6g.kg ⁻¹	+36%	[160]
	0,27g.kg ⁻¹ .h ⁻¹	+73%	[113]
	0,087.kg ⁻¹	+100%	[182]
Leucine	0,1.kg ⁻¹ .h ⁻¹	+50%	[179]
	5g	+100%	[123]
Soja	20,1g	+90%	[155]
	19g	+50%	[169]

humans by Roger Harris [140]. A meta-analysis [141] and some reviews [139, 142] concluded to positive effects while looking to intensive short-time (1–4 min) exercises. The same positive effect was obtained in young athletes submitted to plyometric exercises (45 vertical jumps) following β -alanine supplementation (5 g per day) during a period of 2 months [143]. The same positive conclusion was observed after 800 m run [144] and repeated isokinetics contractions [145]. Moreover, association of sodium bicarbonate (10 mg.kg⁻¹ body weight) to β -alanine supplement (6.4 g day⁻¹) is increasing the performance of a Wingate test (4 times 30 s) [146]. However, it must be emphasized that β -alanine supplementation higher than 10 mg.kg⁻¹ body weight may provoke serious irritations (paresthesia) in some individuals!

- (c) Taurine: This natural human product, derived from cysteine, contains about 10% of sulphur in a single organism, mainly abundant in the heart, muscles, kidneys, brain and retinas [3]. This compound is essential in pre-mature kids to stimulate the development of those cited tissues. In adults, taurine has a potential effect on Ca²⁺ uptake by the sarcoplasmic reticulum of fibres I and II [147]. Several authors proposed to athletes a diet supplement of 2–4 g of taurine to fight against sleep-inducing effect of intense eccentric contractions [148–150]. In opposition to previous authors, other scientists did not confirm any potential positive effects of β -alanine in healthy athletes [151–154].

To summarize the practical use of taurine in healthy athletes, let us compare some evidence offered by some commercial products:

	Mean human diet [13]	Taurine (some commercial product) (355 ml)
Taurine	60 mg 24 h ⁻¹	1 g!
Caffeine (max)	400 mg 24 h ⁻¹	114 mg

Make your choice...!

Proteins of vegetal origin

A few recent publications did analyse the effects of proteins from vegetal origin as supplementation to food intake in order to simulate anabolic response of the human skeletal muscle [155, 156]. Indeed, this information could foster the interest of vegetarian athletes or even vegan individuals. Indeed, the “protein digestibility-corrected amino acid score” (PDCAAS) used by nutritional scientists indicates a higher score for milk, whey proteins and eggs (value 1), as compared to oatmeal

(0.57) or wheat (0.45). Moreover, the concentration of leucine differs in different brands: whole milk (10.9%), whey proteins (13%), oatmeal (7.7%) and wheat (6.8%) [156].

Conclusions

Using the net nitrogen balance, the Institute of Medicine estimates adult protein requirement to a mean of 0.80 g.kg⁻¹ body weight per 24 h. However, those recommendations are focussed on individuals with moderate-intensity physical activity. For strength athletes, the daily amount of protein should represent between 12 and 15% of the total energy requirement. We are convinced that an appropriate diet survey should be applied regularly, together with nitrogen-balance assays, to evaluate the real daily need for protein intake (mean 1.25 g.kg⁻¹ body weight per 24 h) to compensate the exercise training muscle protein degradation and resynthesis. As suggested from previous publications (see above), a bolus of 20–25 g protein drink may be needed immediately after stopping the exercise to stimulate skeletal muscle protein turnover.

Omnivorous and vegetarian athletes need a regular verified food intake to equilibrate whole sorts of protein feeding (types and quantities) to assure optimum quantities of essential amino acids. An excess of protein intake is inaccurate and costly. It appears that vegan athletes should have appropriate advices from a nutritionist/dietician to avoid any unbalanced diet, as recently suggested by a joint position statement of the American College of Sports Medicine and the Academy of Nutrition and Dietetics Dietitians of Canada [157].

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Authors' contributions

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